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THE STRUCTURE OF THE STARCH GRAIN

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Although starch is one of the most common and most widely distributed, as well as one of the most widely used, of organic substances, there is amazingly little known about it. During the past two centuries fully five hundred investigators have worked with starch, and yet there are only a few points which seem to be at all well established. In 1836, Poggendorff (1), in his review of the work done on starch, wrote that "no substance has been investigated more and is still less known"; Carl Nägeli (2) in 1858, Arthur Meyer (3) in 1895, and E. T. Reichert (4) in 1913, in their large monographs express a similar view; and a reviewer of 1921 would very probably arrive at the same conclusion.

One commonly thinks of starch as a product of corn, wheat, and other cereals; or of the potato tuber, the cassava root, or the stem of the sago palm; and recognizes it as a product of plant life, and as the principal form in which food is stored.

Commercially it is separated by grinding the plant tissues in which it is stored and allowing it to settle out from a water mixture. When washed and dried the white powdery mass is the starch of commerce.

Under the microscope this powder is seen to consist of minute, transparent, glossy beads, varying in size from $1/10$ of a millimeter down to the limit of visibility, that is, about $4/10,000$ of a millimeter in diameter. They vary also in shape, although the general tendency is towards a rounded, approximately spherical form. On close examination in water they appear to consist of a series of more or less concentric layers, or lamellae. In polarized light a dark cross appears in each grain. If, however, the grains are made to swell by treating them with hot water, or with certain chemicals, the dark cross disappears.

The natural whole grains seem to be insoluble in cold water; but if treated with hot water or if the individual grains are crushed, they swell and appear to go partly into solution. The air-dried grains usually contain a high percentage of moisture, often up to 20 percent.

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PROBLEMS PRESENTED BY STARCH

These statements hint at a few problems which in turn lead to many more. At this place, however, there is no need to do more than to give briefly the status of the larger groups of these problems, in order to locate, in the whole field of the work on starch, the position of that on the structure of the grain.

About the first problem which attracted the early investigators was that concerning the identity of the components, or compounds, which make up the starch grain. Starting with Leeuwenhoek (5) in 1719, many investigators have attempted to solve the problem, but up to the present time, the scores of contradictory papers on the subject lead one to conclude that the correct answer is not yet firmly established. Many methods have been tried and repeated, but there is still considerable uncertainty whether starch consists of a single substance or of two or more substances. Perhaps it is the ease with which starch becomes modified that is the cause of so much uncertainty.

The place of origin of the starch grain is more certain. It has been verified many times and can be considered as settled. Crüger (6, p. 46) in 1854 pointed out that all starch grains originate in the protoplasm of the cell; Schimper (7) in 1880 reported his discovery of the unstable albuminous bodies which always accompany starch grains; and Strasburger (8) a few years later put on a firm basis the statement of the earlier workers, that starch grains are formed inside plastids (protoplasmic bodies)—chloroplasts in the green parts and leucoplasts in those parts which are not green. He reports cases, however, in which these bodies are not differentiated from the rest of the protoplasm.

The mechanism of the formation of the grain is not known. Only one thing seems certain; that is, that the grains grow only where they are in contact with plastids.

Many of the chemical and physical properties have not yet become fully established. About the only one which seems quite satisfactory to the chemists is the percentage composition. They have accepted $C_6H_{10}O_5$ as reported by Guérin Varry (9) in 1834, but whether a dozen or a hundred of those groups constitute a molecule is not at all certain. The formula, therefore, is written $(C_6H_{10}O_5)_n$, and the molecular weight is often written " n 162.1." The solubility of starch is uncertain and the specific gravity is variable. The color reaction with iodine solution varies greatly with the conditions of the experiment. The reactions with acids and enzymes lead into a large field of uncertainties, which, however, have little or no direct bearing on the present work.

STRUCTURE OF THE GRAIN

The physical structure of the grain has been a subject of much discussion during the past hundred years; and since this paper deals with one of the

many problems involved in determining the physical structure, this phase will be described in considerable detail.

The grain appears to have a layered structure somewhat analogous to that of an onion, that is, sphere inside of sphere. Strictly speaking, however, the more truly spherical layers occur only near the center; the other layers are generally thicker on one side, making the shape of the larger grains eccentric.

One group of investigators, working primarily with swollen grains, considered the grain as a sac composed of a different substance from the enclosed starch. The lamellar structure was more or less ignored. Leeuwenhoek (5) and Raspail (10) were among the earlier members of this group, and Whymper (11) and Beijerinck (12) are among the present-day representatives.

A smaller group report evidence sufficient to warrant the supposition that there is a series of concentric vesicles representing layers of the grain. Maschke (13), W. Baily (14), and Mme. Z. Gatin-Gruezewska (15) were advocates of this theory.

By far the greater number of investigators have considered the layers as essential parts of the structure of the grain. That the layers differ in composition was held by Maschke (16), Nägeli (17), Meyer (18), Kraemer (19), and Gatin-Gruezewska (15). That they differ physically, perhaps dense and less dense layers alternating, was favored by Fritsche (21), Münter (22), Kabsch (23), Sachs (24), Strasburger (25), and de Vries (26).

The possibility of a crystalline structure of the grain was first brought out by Raspail (27) in 1825; but on account of its existence fully formed and free in plant cells, of its glossy round form, its insolubility in water, its color reaction with iodine, and its decolorization with alkali, he decided it could not be a crystal structure.

Münter (28) considered that he had proved the grains to be crystalline, although they could not be recovered in crystalline form, when he discovered that they would dissolve in sulphuric acid and that the solution would turn blue with iodine.

Whatever these investigators may have had in mind concerning the structure of the crystal, it remained for Nägeli (2) in 1858 to develop the first theory which was to hold a large place in the literature on the minute structure of the grain. He conceived the layers to be composed of invisible particles, and developed this theory along with his intussusception hypothesis of the growth of the starch grain in his monograph "Die Stärkekörner." It is a rather complex theory in that there are many assumptions concerning both the particles and the tensions and forces involved. In brief, the theory is as follows (2, pp. 332-377): The layers of the grain are built up of small particles of starch, the largest of which is invisible with the most powerful microscope, yet it contains over 9000 molecules of starch. These particles, which he later calls micellae (29), are surrounded by a water-shell

which is thin on the larger particles and thick on the smaller particles. They may consist of either of the two substances, granulose or cellulose, which Nägeli considered to be the two components of starch. The denser layers of the grain are composed of the larger particles while the less dense or softer layers contain only the smaller particles with the thick water-shell.

He supports this construction by long discussions of the forces which must be exerted to account for various starch reactions with water. In several places he said these particles are not crystals, but in 1881 (29) he stated that the micellae are of a crystalline nature.

About thirty years after Nägeli's theory appeared, Schimper (30) undertook to determine whether the starch grain is crystalline or amorphous. He decided that the cohesive and the optical properties would differentiate these two states. From his work with polarized light he concluded that the grains must consist of fibrous crystals arranged at right angles to the concentric layers; and that they differ from the ordinary spherocrystal through their ability to swell in water.

In 1895, Arthur Meyer (3) in his large monograph, "Untersuchungen über die Stärkekörner," accepted, to a great extent, the conclusions of Schimper and elaborated the spherocrystal conception of the starch grain. He used the definition (3, pp. 101-107) of the spherocrystal which Nägeli (31) and Rosenbusch (32) proposed—that it is a more or less globular body composed of radially arranged needle-like crystals. Fox Talbot (33) in 1836, Sir David Brewster (34) in 1853, Rosenbusch (32) in 1885, and others had studied the optical properties of globular crystal aggregates in which they could see the needle-like crystals arranged radially from a central point. The dark cross produced by polarized light seemed to be the same in them as that formed in other globular bodies in which the individual crystals were invisible. The latter, then, they reasoned, consisted likewise of invisible radially arranged crystals. In addition to this, they found spherical forms which presented gradual transitions from those with visible crystals to those in which the crystals were not visible.

Basing the conclusion primarily on this work, Meyer (3, pp. 116-129) felt justified in assuming that the starch granule is a spherocrystal. The invisible needle-like crystals he called trichites. He decided that the concentric layers of the grain are visible because the trichites differ in size and number in the different layers. By comparing the properties of inulin and amyloextrin spherocrystals with those of the starch grain he arrived at the following conclusions:

1. In the starch grain radial lines of weakness occur just as they do in the spherocrystals, where they are due to radially arranged trichites.

2. Optically the spherical grains behave exactly like the spherocrystal, while the eccentric forms behave as though they were built up of trichites placed at right angles to the concentric layers of the grain. Very small broken pieces also behave as though composed of trichites.

3. The concentric layers in the spherocrystals are visible because the space between the trichites varies. This might be the cause in the starch grain also.

4. The layers are due to periodic changes in the mother-liquor, which affects the growth and form of the trichite in both the spherocrystals and the starch grains.

These comparisons and conclusions, based to a great extent upon experiment, led Meyer to conclude that the grain is a spherocrystal. Only a few investigators have opposed this theory; many have accepted it.

H. Fischer (35) found the dark-cross phenomenon in hardened mucilage of certain orchids, and Strecker (36) found the same effect in the guard cells of the stoma. Czapek (37) considered both of these cases to be due to symmetrically distributed tensions and inferred that the dark cross of the starch grain might be due to similar causes.

Reichert (4, p. 82) in his large monograph on starch accepts the spherocrystal conception without question.

Perhaps the latest bit of evidence in this connection is from work done with X-rays. Herzog and Jancke (38) reported a crystalline structure for both cellulose and starch, although no details of their work with starch were given. The method they used is one by which it is possible to determine whether a powdered substance has an amorphous or a crystalline structure; also, if the latter, to determine the crystal system, although in certain systems the problem becomes very complex. Further, in many instances it is possible to determine the location of the different kinds of atoms which make up the crystal.

That method is the one used for the experimental work which was done for this paper, and is described in detail below.

THE X-RAY METHOD OF DETERMINING CRYSTAL STRUCTURE

The method consists, briefly, in photographing the X-rays which are reflected from the various atomic planes of a crystal. In order to explain this method it may be of advantage to have some of the concepts which will be used described in terms of present-day science.

The Crystal

Since 1850, the face of a crystal has been considered an indication of its internal atomic structure (39). No direct evidence of this was brought forward until Laue (40) in 1912 conceived the idea that if X-rays are actually electromagnetic waves, they should show interference phenomena when reflected from a crystal, since the size of the waves approaches the magnitude of the distance between the atoms of a crystal. He worked out the mathematical part of the problem, and Friedrich and Knipping (41) proved experimentally the correctness of his figures. From this brilliant beginning the structure of many crystals has been determined; resulting in the

present-day concept of a crystal as an arrangement of atoms so placed that all of them lie in planes which are regularly spaced. The cubical crystal of common table salt will serve as an illustration. One could think of the structure of such a crystal as somewhat like that of a cubical box exactly filled with equal-sized oranges, each orange representing an atom. The oranges would arrange themselves in layers, and no matter whether the box were standing on a flat side or balanced on an edge or on a corner, there would be, horizontally, layer above layer. When the box is standing with a flat side on the floor, the distance between the center planes of the layers will be, say, 1 unit. If the box is balanced on an edge, the distance between the new horizontal planes will be less than the unit; actually it will be $\frac{1}{2}\sqrt{2}$ (or .707) times the unit. If the box is balanced on a corner, then the planes which are now horizontal will be still closer together, actually $\frac{1}{3}\sqrt{3}$ (or .577) times the unit. In each case the distance between the atoms of a single plane becomes greater. That is, as the planes become closer together the atoms in the planes become farther apart. There are many other planes, but there is no need of further discussion of them here. Of course, the atoms may be relatively farther apart than the oranges in the illustration, leaving considerable space between them. One can visualize this arrangement as a sort of lattice with the atoms at the corners, a three-dimensional lattice. The expression "space lattice" is commonly used in this kind of work.

In mineralogy there are several ways of designating the planes described above; the system generally used in the X-ray work with crystals designates the first plane as the 100 plane, the second as 110, and the third as 111. These figures refer to a relation between the planes and the axes of the crystal. More detail concerning the crystal is probably not necessary here.

X-Rays

The modern conception of the atom is that it consists of a core, or nucleus, surrounded by a system of electrons, which are situated at a considerable distance, relatively, from the core. The electrons may be arranged in concentric rings, the number of both electrons and rings varying with the different elements. One or perhaps several of these may be shot off from the atom by appropriate means without changing the elemental nature of the atom.

If a high-voltage electric current is allowed to pass through a vacuum tube which has at its cathode a fine wire spiral at white heat, a stream of electrons will be shot off from the spiral to the anticathode. They will attain a velocity somewhat less than that of light. If this stream of electrons is allowed to impinge on a metal anticathode, the atoms of that metal will be jarred into vibration as the electrons hit them. This vibration starts a series of waves in the surrounding ether, from each atom. These waves are X-ray waves. The wave-lengths are of the order of the Ångström

unit (1 Å. (Ångström) = $\frac{1}{10,000,000}$ mm.). Every metal produces more or less of all wave-lengths. These can form a spectrum comparable to that of white light. Further, each metal produces a few wave-lengths which are characteristic of itself and which have an intensity far in excess of the others; just as in light, each element has its characteristic lines. The characteristic wave-lengths produced by a rhodium anticathode, such as was used in the experimental work which is to be described below, are .617 Å. and .533 Å. in length. The energy conveyed by these waves is not carried in equal amounts by all wave-lengths, usually one or two carrying almost all of it. Of those from rhodium the .617 Å. wave-lengths carry perhaps 70 percent of the total characteristic ray energy, while the .533 Å. wave-lengths carry perhaps more than half of the remainder.

A beam of such waves, when reflected from a crystal, will be sorted out into a spectrum of several lines which may be caught on a photographic plate. The line due to the .617 Å. wave-lengths will be much darker than the others, and is called the α line. The .533 Å. line, called the β line, will be much less intense, and a third line, the γ line, will be still fainter. The first two are all that are of importance here. In fact, the lines obtained in the present set of experiments are all α lines, with the exception of a few faint β lines. The "white light" or general radiation, as it is sometimes called, merely causes a general darkening of the negative. It follows that the beam, for purposes of explanation and for coarse work, may be considered as consisting of only the .617 Å. wave-length.

The statement that the waves are "reflected" is not quite true, but the end results are comparable to those of light reflection so that the idea is conveyed by the word. A light wave, which may be 10,000 times the length of an X-ray wave, will be wholly reflected from a layer of atoms such as the surface layer of a crystal, while the short X-ray wave will be only partly reflected; in fact, only a very small part is reflected from a single plane. By far the larger part passes through to the next plane, where a minute portion is again taken from it and reflected, and the remainder passes on to the next plane beneath, continuing in this way for perhaps a million planes each reflecting only a minute portion.

The so-called reflection is brought about in this way: when a wave hits an atom it sets the atom to vibrating, and that atom in turn produces a secondary set of waves of the same kind. These secondary waves from a plane of atoms will form a wave front which will leave the plane at the same angle at which the primary beam strikes it; that is, the angle of incidence is equal to the angle of reflection. It is readily demonstrated geometrically that these secondary waves, produced by planes of atoms, will reinforce each other, resulting in a strong wave front when a certain relation exists between the wave-length, the distance between the planes of atoms, and the glancing angle of the primary beam. This demonstration

was made and is clearly explained by Bragg and Bragg (45). The relation which is fundamental in all work of this sort is expressed by the equation

$$\lambda = 2d \sin \theta,$$

where λ is the wave-length, d is the distance between the planes of atoms and θ is the angle between the planes and the beam of X-rays. From these relations it follows at once that where only one wave-length and only one set of planes is used, the crystal will produce "reflections" only when in very definite positions.

These points become clearer perhaps with a little study of figure 1.

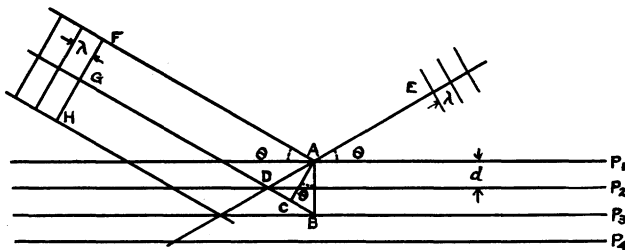


FIG. 1. From "X-ray and crystal structure," by W. H. Bragg and W. L. Bragg.

If P_1 , P_2 , P_3 , P_4 represent the planes of a crystal, planes perpendicular to the paper, and FGH a wave front moving towards these planes, a part of the wave front reflected from plane P_1 follows the path AE and a part reflected from P_2 will likewise fall into the path AE extended from D , and similarly from plane P_3 , etc. Now in order to have the wave from P_2 reinforce that from P_1 , *i.e.*, to have crest coincide with crest, the wave from D must be exactly 1 wave-length, λ , behind that from A . In other words, the part of the wave from F must travel through a distance 1 wave-length less than the part from G , or

$$FA + AE \text{ must equal } GD + DE - \lambda.$$

Without going into the details of the proof, it happens that CB is the difference between the distances traveled by F and G to reach E ; and CB then, if the waves reinforce each other, must be equal to λ . It can easily be shown that the angle opposite CB is equal to the angle θ formed by the beam and the planes, and since $AB = 2d$, then,

$$\begin{aligned} CB &= AB \sin \theta, \\ \text{or} \\ \lambda &= 2d \sin \theta. \end{aligned}$$

If the difference between the distances traveled by F and G to reach E is exactly equal to λ , then the wave from P_1 will be reinforced not only by

that from P_2 , but also by the waves from P_{1000} , and from any other plane the rays can reach. But if that distance is greater or less than λ , by even one part in 2000, for example, then the wave from P_{1000} will neutralize that from P_1 ; and the one from P_{1001} will neutralize that from P_2 , and so on. The neutralizing effect is not quite complete, because the incident rays reaching P_{1000} are weakened by passing through so many planes. So it happens that unless λ is almost exactly equal to $2d \sin \theta$, reflection will fail to occur. It is important to notice that this conclusion is based on the assumption that the reflecting body consists of a large number of planes. If, on the other hand, the number of planes from which reflection occurs is small, then the neutralizing effect becomes less complete and the reflected line becomes broad and blurred; and an unusually large range of angles will be effective in producing the reflected line.

Reflection from Crystal Planes

In discussing the structure of the crystal a few pages back, three prominent sets of planes were mentioned. Those parallel to the faces of the cube, which were called the 100 planes, were separated by a uniform distance d . If that crystal were placed so that a narrow beam of X-rays hit these faces making the glancing angle equal to θ_1 , then a reinforced train of waves would be reflected from them. If it were placed so that the angle were slightly greater than $2\theta_1$, another reinforced train of waves would be reflected, and the equations would be

$$\begin{aligned}\lambda &= 2d \sin \theta_1, \\ 2\lambda &= 2d \sin \theta_2, \\ 3\lambda &= 2d \sin \theta_3, \text{ etc.}\end{aligned}$$

These are reflections of the first order, second order, third order, respectively. The subscript figure is used to designate the larger angles and incidentally the order of reflection. The second-order lines are very weak, and those of the third order are still weaker. The latter are not considered in the present work.

If the crystal were placed so that the beam made the proper glancing angle with the 110 set of planes, then again a reinforced reflection would occur. The distance between these planes is $\frac{1}{2}\sqrt{2}$ times the distance between the 100 planes, so that now d has a new value, and since λ remains the same, $\sin \theta$ must be a new value in the equation, $\lambda = 2d \sin \theta$. Another change in values takes place when the 111 planes are in position to reflect the beam, and again d and θ would have new values. The equations would read for the first-order reflections:

$$\begin{aligned}\text{For 100 planes, } \lambda &= (2d \times 1) \sin \theta_1', \\ \text{For 110 planes, } \lambda &= (2d \times .707) \sin \theta_1'', \\ \text{For 111 planes, } \lambda &= (2d \times .577) \sin \theta_1'''.\end{aligned}$$

The accent marks indicate different values of the angles which produce first-order reflections.

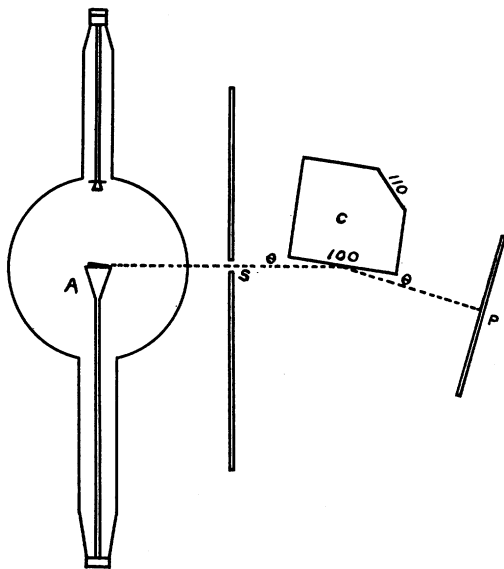


FIG. 2. X-ray tube with crystal in position.

A diagram of an X-ray tube with a crystal in position (fig. 2) may make this somewhat clearer. *A* represents the anticathode which is the source of the X-rays. *S* is a lead screen which absorbs all the rays except a beam which a slit allows to pass through. This beam is incident on the crystal *C* from which the reinforced waves are reflected to the photographic plate *P*, where they produce an effect resulting in a black line when the plate is developed. In the position shown, the rays are incident on the 100 planes. If, without disturbing the tube and the plate, the crystal was moved so that the glancing angle θ would be increased the proper amount, then a line of the second order would be produced from the same planes. If still further changes were made, without disturbing the tube and the photographic plate, the two orders of reflection from each of the two other planes, 110 and 111, would be thrown on the plate, one at a time. Since each reflection has a definite glancing angle which is different from the others, there would be produced on the plate six lines.

It seems perfectly plausible that six small crystals could be put in place of the one large crystal, *C*, and be so arranged that one would produce the first-order reflection from the 100 planes; another one so that it would produce the second-order reflection from the same planes; a third so that it would produce the 110 first order, and a fourth the second order; the remaining two could produce the first and second orders from the 111 planes. With this arrangement all six lines could be photographed at the same time.

It is only a step further to break those six crystals into many smaller crystals, since the crystal structure cannot be destroyed by that means, and to pack them in a small tube of some amorphous substance, like glass or celluloid; to allow the beam of X-rays to pass through this tube; and to photograph the many minute reflections which will be produced by this random arrangement of tiny crystals. Since all of those small crystals which have their 100 planes at the proper angle of incidence to the beam will send their reflections out at the same angle, the sum of these small reflections will make a large line on the photographic plate; this will happen also with each of the other planes for both first- and second-order reflections; and as a result the photographic plate will have all six lines which might have been obtained by carefully manipulating a single large crystal or by carefully arranging six smaller crystals.

From this it is just another step further in thought to realize that a powder of an unknown structure might be substituted for the known powder, and certain facts concerning its atomic structure be obtained from the photographic plate.

Based, more or less, upon this sort of reasoning, Hull (42) and Debye and Scherrer (43) independently devised methods, which are very much alike, for studying the atomic structure of crystal powders. Since then several modifications have been made in the refinement of the apparatus, but the essential features have remained the same. They also determined certain conditions which must be complied with for the success of the method, such as the most suitable X-ray tube, and the best voltage, connected with the production of the rays; the fineness of the powder which will give the clearest lines, and the kind of containers for the powder. Through their work and that of others, means have been found for obtaining almost pure monochromatic beams, that is, one wave-length only; and for increasing the effect on the photographic film by converting the waves which have passed through the film into light waves.

The experimental part of the work for this paper was done with an apparatus built on the principles laid down, for the greater part, by Hull (42, 46).

EXPERIMENTAL PART

Apparatus

The apparatus (fig. 3) consists of two parts: an X-ray tube (its high-voltage electrical equipment not shown) and the photographic part. The X-ray tube, *X*, is enclosed in a lead-covered box, *B*. The photographic film, *F*, is placed on the circumference of a flat, semicylindrical film-holder, *H*. The powder to be investigated is pressed into a container, *P*, which is located at the intersection of all the radii from the film. The beam of X-rays from the anticathode, *A*, passes through two adjustable lead slits, *S*, *S*, and into the powder where a small part of the beam is reflected to

the film at various definite angles while the larger part passes through the powder and is absorbed by the lead shield, *L*, just before it would otherwise reach the film. Concerning the details of construction only enough will be

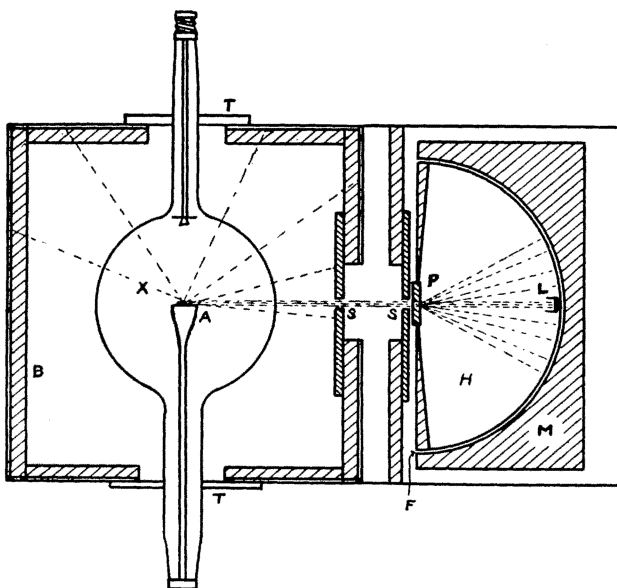


FIG. 3. X-ray tube and film-holder viewed from above.

described to supplement figure 3 in making the experimental work clear. Several detailed descriptions, which vary only slightly, are in the literature (42, 43, 44, 46).

A Coolidge tube with a broad-focus rhodium anticathode was used as the source of the X-rays. The bulb of the tube is 18 cm. in diameter. The box which incloses the tube is approximately 30 cm. on each side and is covered with sheet lead 2 mm. thick. The tube is supported by sheets of asbestos, *T, T*. The lead slides, *S, S*, are about 2 cm. high and adjustable to form a slit of any width.

The film-holder, *H*, is a hollow, semicylindrical box 5 cm. deep with a radius of 14 cm. The block, *M*, which fits around it is of solid wood and also 5 cm. deep. The film lies between these two parts. The film-holder, *H*, is made from three semicircular pieces of sheet lead. One forms the top, one the bottom, and the third divides the box into halves, an upper and a lower. They are held in place on the straight side by two pieces of wood and on the curved side by the lead shield, *L*, which is about 5 mm. thick. Without this absorbing shield the film would become heavily fogged at this place. The whole of the curved side of the holder is covered with black paper.

The container for the powder is merely a piece of sheet lead, about 3

mm. thick, with two windows cut through it, one above the other, into which the powder is pressed just firmly enough to support itself so that no covers are needed. When in position (at *P*) each window opens into one compartment of the holder and so provides for making photographs of two powders at the same time.

When the apparatus is ready for exposure, the two adjustable slits are opened only enough to allow a narrow, ribbon-like beam of X-rays to pass through. This beam is about 1 to 2 mm. wide and 20 mm. high. When it strikes the powder container, the upper half passes through the powder in the upper window and the lower half through the lower window. The part of the beam which goes straight through strikes the lead shield, *L*, and is absorbed; while the part which is reflected by the tiny crystals meets but little resistance in the black paper and so reaches the film. The rays which react on the film fall into two categories: the first contains the reflected characteristic rays which form the lines; the second contains those rays which produce the general darkening of the film between the lines. The rays of the second category are in part reflected "white light," or general radiation, and in part from any amorphous material present; both will cause a diffuse scattering like that produced on light by smoke or fog. Rays of the first category only can produce the lines and so indicate the presence of crystalline matter. The lines will be approximately the width of the lead slits, *S*, *S*.

In this type of film-holder the primary beam cuts the film into halves which, so far as the lines are concerned, are mirror images. There are at least two advantages arising from this: (1) since the lines must appear in each half, the possibility is eliminated of mistaking a developer flow line; and (2) the error in measuring the arc of the glancing angle is reduced, since the distance between pairs of similar lines is four times the length of the arc.

The electrical part of the apparatus was calibrated so that the voltage could be kept at approximately 40,000 volts and the current at from 3 to 5 milliamperes.

The powder for examination was ground fine enough to pass through a sieve having 200 meshes per inch. It was found that if the particles were too large to go through such a sieve, the lines produced on the photographic film were composed of large spots; but if the particles are small enough to pass through, the spots are invisible and the lines appear solid.

Each sample was pressed into the lead slide with just enough pressure to make a tablet-like mass which would be solid enough to support its own weight. The thickness of this tablet would vary with the substance used, for there is a fairly definite relation between the atomic weights of the elements in a substance and the absorption of X-rays by it. For example, the elements in salt are almost twice as heavy as those in starch, so the tablet of starch which would allow the maximum amount of reflected

energy to pass through to the photographic film would be approximately twice as thick as the most efficient salt tablet.

Eastman's duplitized X-ray films were used, two films being used for each exposure. The two films made it possible to observe very faint lines which would not be distinguishable on a single film.

Test Reflections

In order to test the apparatus, sodium chloride was used first, for its crystal structure has already been worked out and it is known to produce lines readily by this method. An exposure of 4 hours, at 40,000 volts and 4 milliamperes current, produced the lines shown in figure 4.

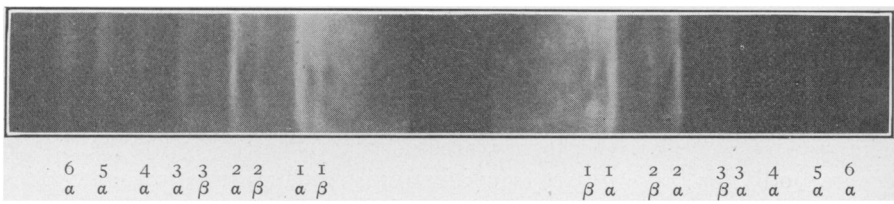


FIG. 4. Sodium chloride, positive print. Reflected lines are light.

If these lines corresponded favorably with their calculated position, then the apparatus could be expected to produce lines from other crystal powders. Also the denser lines, those numbered 1_a, 2_a, etc., should correspond with the theoretical position of the lines made by reinforcement of the waves having a .617 Å. wave-length; and the fainter lines, numbered 1_β, 2_β, 3_β, should be in the positions where reinforcement occurs of the waves having a .533 Å. wave-length. The latter carry much less energy and would therefore make fainter lines.

Tables 1 and 2 give the results of measurements of the glancing angles, and of the computations based on the equation, $\lambda = 2d \sin \theta$, which shows

TABLE 1. Lines Produced by Sodium-chloride Powder; Rhodium Anticathode; $\lambda = .617 \text{ \AA}$.

No. of Line α Lines	Distance between Lines (mm.)	Glancing Angle θ^*	Sin θ	Distance between Atomic Planes $d = \frac{1}{2} \left(\frac{\lambda}{\sin \theta} \right)$	d Calculated	Ratio	Indices of Planes
1	61.5	6° 18'	.10973	2.81 Å.	... Å.	1.00	100
2	87	8° 54'	.15471	1.99	1.99	.707	110
3	107	10° 57'	.18995	1.63	1.62	.577	111
4	122.5	12° 32.5'	.21715	1.41	1.41	.500	100†
5	138	14° 7'	.24390	1.26	1.26	.447	210
6	152	15° 34'	.26836	1.15	1.15	.408	211

* The film, when in the film-holder, lies on the circumference which subtends all of the glancing angles. By construction, 1° on this circumference equals 2.44 mm.

† This line is the result of reflection of the second order from the 100 planes; and here, d should be exactly $\frac{1}{2}d$ as obtained from the reflection of the first order from those planes.

the relation between the wave-length, the distance between planes of atoms, and the glancing angle θ . The tables also give, for comparison, the calculated distances between planes based on the assumption that line 1 was produced by the 100 planes of the crystals.

TABLE 2. *Lines Produced by Sodium-chloride Powder; Rhodium Anticathode; $\lambda = .533 \text{ \AA}$.*

No. of Line β Lines	Distance between Lines (mm.)*	Glancing Angle θ	Sin θ	Distance between Atomic Planes $d = \frac{1}{2} \left(\frac{\lambda}{\sin \theta} \right)$	d Calculated	Ratio	Indices of Planes
1	53	$5^{\circ} 26'$.09469	2.81 \AA \AA .	1.00	100
2	76	$7^{\circ} 47'$.13543	1.96	1.99	.707	110
3	95	$9^{\circ} 35'$.16648	1.60	1.62	.577	111

* These lines are so faint that close measurement is not possible.

Since the actual and calculated values are so close, two inferences may be drawn: first, that any lines produced by another powder, in place of the salt, must be reflected by the same sort of planes, *i.e.*, planes of atoms; and secondly, that the principal lines will be α lines.

It is possible to cut out the β lines completely or practically so by using a filter made from the metal, or a simple salt of the element, which occurs in the periodic table one or two places below the metal used in the anticathode. In the case of a rhodium anticathode the filter would be made of ruthenium. But the filter reduces the intensity of the lines perhaps 50 percent, and this would mean a considerable increase in the time of exposure. Since, however, the salt photograph shows that the principal lines are the α lines, the preliminary work can be carried on without using a filter.

There is supposed to be a relation between the intensity of the secondary waves produced by an atom and its atomic weight. On this account, it was thought necessary to try a carbohydrate known to be crystalline, such as cane sugar, in order to determine the best conditions for getting reflections from carbon and oxygen atoms. The upper half of figure 5 shows

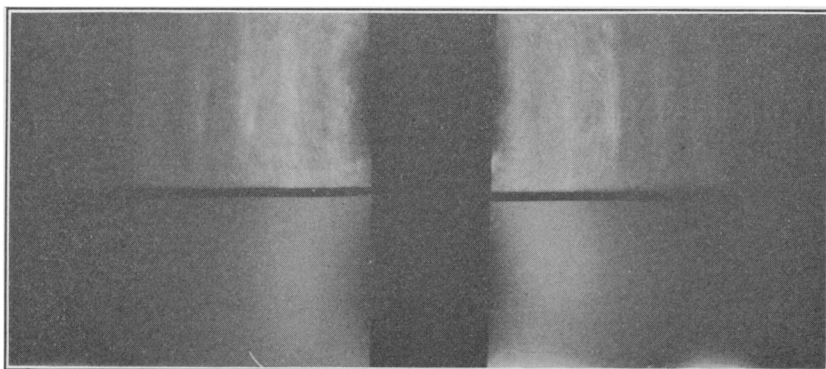


FIG. 5. Above, cane sugar; below, dextrine.

the results obtained, and it seemed from this that any other carbohydrate in which there was uniform atomic arrangement would also give lines due to reinforcement of the reflected waves. If, however, the atoms were not uniformly arranged, lines should not appear, and in order to demonstrate this, another carbohydrate, dextrin, which is generally considered amorphous, was photographed along with the cane sugar. An exposure of 18 hours was given. The lower half of figure 5 shows the picture obtained from this amorphous substance, in which the rays are merely scattered, not reinforced at any single place. The atoms in air give the same sort of

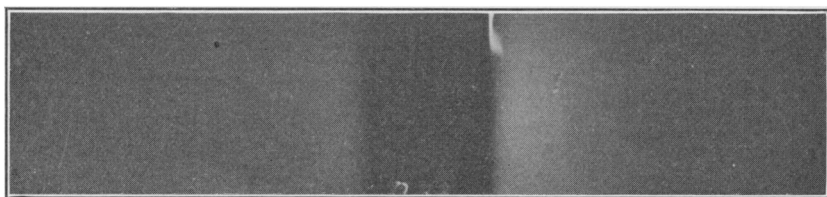


FIG. 6. Air.

scattering, but much less intense, as shown in figure 6, with an exposure of 18 hours.

Reflections from Starch Grains

After these preliminaries, in which it was shown that the apparatus would produce lines with a crystalline, and none with an amorphous, substance; that the principal lines were α lines; and that the exposure for

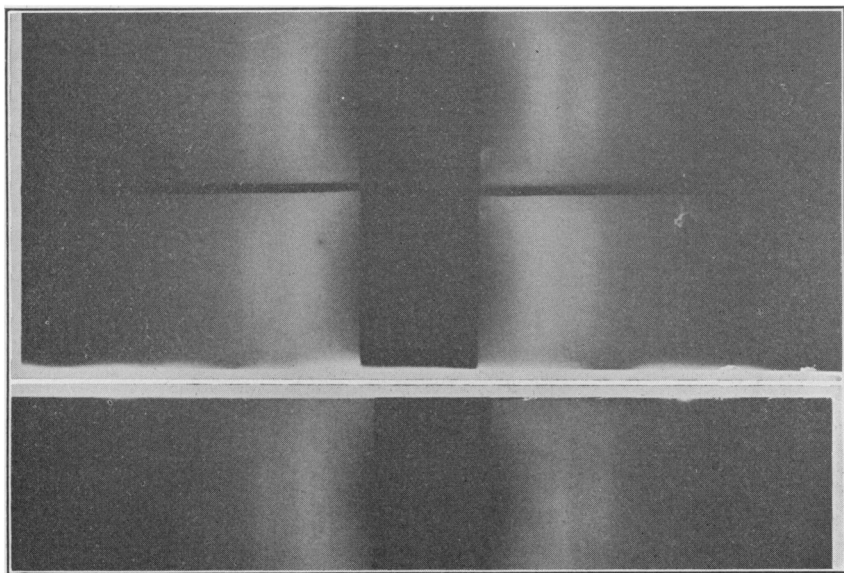


FIG. 7. Above, potato starch; middle, cassava starch; below, corn starch.

carbon and oxygen atoms was about 18 hours; three different kinds of starch were photographed: corn, potato, and cassava.

The dry powder consisting of whole grains of starch was pressed into the specimen-holder just firmly enough to support itself. Potato and cassava starch were taken at one time; and corn starch and a blank at another time. The exposures were for 18 hours. The voltage was approximately 40,000, and the current varied between 3 and 5 milliamperes. Figure 7 shows the results of these exposures. The lines are very much blurred, and on that account are difficult to measure. Careful study brought out several more lines on the films than are shown in the prints made from them.

In tables 3, 4, and 5 the measurements and the computed results are given. The computations are based on the equation, $\lambda = 2d \sin \theta$, where $\lambda = .617 \text{ \AA.}$, d is the distance between planes, and θ is the glancing angle determined from its arc which was measured on the photographic film.

TABLE 3. *Corn Starch*

No. of Line	$\sin \theta$	d Actual	d Calculated from Cube	Difference	Indices of Planes of Cube
1a	.05175	5.96 Å.	5.87 Å.	-.09	111 ^(x2)
1	.06210	5.04	100
2	.08020	3.85	3.56	-.29	110
3	.10366	2.98	2.91	-.07	111
4	.11667	2.64	2.52	-.12	100 ⁽²⁾
5	.13399	2.30	2.25	-.05	210
6	.14608	2.10	2.06	-.04	211
7	.15845	1.95	1.78	-.17	110 ⁽²⁾

TABLE 4. *Cassava Starch*

No. of Line	$\sin \theta$	d Actual	d Calculated from Cube	Difference	Indices of Planes of Cube
1	.06076	5.08 Å.	... Å.	...	100
2	.08020	3.85	3.59	-.26	110
3	.10366	2.98	2.93	-.05	111
4	.11494	2.69	2.54	-.15	100 ⁽²⁾
5	.13053	2.36	2.27	-.09	210

TABLE 5. *Potato Starch*

No. of Line	$\sin \theta$	d Actual	d Calculated from Cube	Difference	Indices of Planes of Cube
1	.05902	5.20 Å.	... Å.	...	100
2	.07962	3.88	3.68	-.20	110
3	.10366	2.98	3.00	+.02	111
4	.11667	2.64	2.60	-.04	100 ⁽²⁾
5	.13139	2.35	2.32	-.03	210
6	.14608	2.10	2.12	+.02	211

Before any conclusions are drawn from the tables it must be understood,

first, that the lines from no. 3 to no. 7 are so dim that their measurements must be rather uncertain, and secondly, that the error in measurement is large because of the width of the lines and of their blurred condition. How large this error may be is shown by several sets of measurements given in table 6, taken under very favorable conditions of light and at different times, four to six measurements of each starch.

TABLE 6

Distances between Planes for Line 1

Corn starch varied from	4.99 to 5.08	Average, 5.04
Cassava starch varied from	5.03 to 5.15	" 5.08
Potato starch varied from	5.15 to 5.22	" 5.20

Distances between Planes for Line 2

Corn starch, all measurements	Average, 3.85
Cassava starch, all measurements	" 3.85
Potato starch varied from	3.85 to 3.93

Compared to the figures for the salt measurements given above, these variations are large. But in spite of this fact, there are certain rather constant variations which seem to make the tables worth a few moments' study.

In the first place, there is a marked similarity between the three kinds of starch, also between the starches and the calculated spacings for a cubic arrangement, as shown in table 7.

TABLE 7. *Comparison of d for the Starches and for a Cube Based on the Average for Line 1*

Line No.	1	2	3	4	5
Corn	5.04	3.85	2.98	2.64	2.30
Cassava	5.08	3.85	2.98	2.69	2.36
Potato	5.20	3.88	2.98	2.64	2.35
Cube based on ave. of line 1	5.11	3.62	2.95	2.56	2.29

On the other hand, it is quite certain that there is no nice arrangement of the planes such as in the salt or the sugar crystals. Both the blurring of the lines and the irregular spacing are evidence of that.

In column 5 of tables 3, 4, and 5 are recorded the differences between the actual d and the theoretical d . Line 2 shows a consistently large difference. Line 4 also shows a large difference, although less than line 2. This leads one to suspect that the arrangement is not quite cubic. The blurring of the lines, however, makes the error in measurement too large to warrant further speculation in that direction. Finer and sharper lines are needed before going further.

Another point which determines certain future work is brought out by the no. 1 lines of corn starch. Here the broad line is resolved into two lines, 1 and 1a, while with potato and cassava starches only one broad line is

visible. Refinement of the apparatus will very probably bring out lines which will suggest a different arrangement of atoms than the cubic as assumed up to this point. On the other hand, accepting the evidence as it exists now, line 1*a* may be the β line ($\lambda = .533 \text{ \AA.}$) for the 100 planes, just as line 1 is the α line for those planes. If that assumption is correct, then d in the equation, $\lambda = 2d \sin \theta$, should be the same for both wavelengths. Actually, line 1*a*, using $\lambda = .533$, gives d as 5.02, while line 1, using $\lambda = .617$, gives d as 5.04. The two values for d agree very closely, but with the range of error so large, nothing more than mere suggestion is safe.

There is another possible significance to the line 1*a* which, if verified, may lead deeper into the composition of the layers of atoms. The position of this line is very close to the place where the reflection from the 111 planes would occur if these planes were alternately composed of different kinds of atoms—for example, of layers of carbon atoms alternating with mixed layers of hydrogen and oxygen. Here, again, more refined apparatus is needed.

Quite another set of speculations is started from another photograph. If the starch grain were built up of minute crystals as A. Meyer (3, pp. 116–129) contended, then a mass of crushed grains ought to produce the same lines that the whole grains do. In order to demonstrate the truth or fallacy of this contention, dry potato starch was ground in a pebble mill until samples contained no whole grains. Figure 8 shows the result of

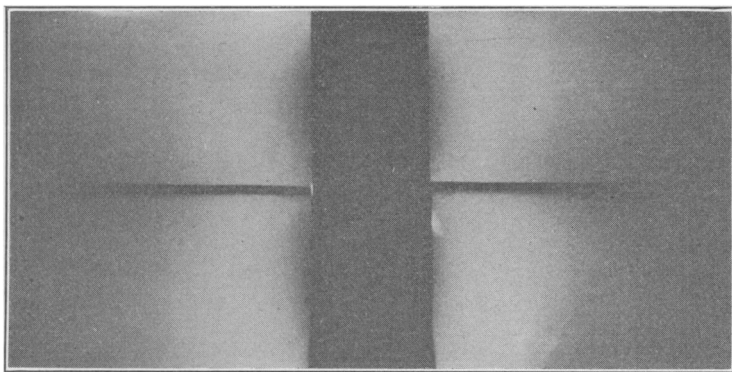


FIG. 8. Above, potato starch, whole grains; below, crushed grains.

photographing, at the same time, the reflections from the crushed grains and from the whole grains. A check photograph gave the same results. Careful study of the films failed to reveal any lines produced by the crushed starch. It does not seem probable that the crystalline structure could be changed into an amorphous form by mere crushing. To test that assumption, cane sugar was ground in an agate mortar until the size of the minute

particles agreed favorably with the size of the crushed starch particles when examined under the microscope. Figure 9 shows the resulting photo-

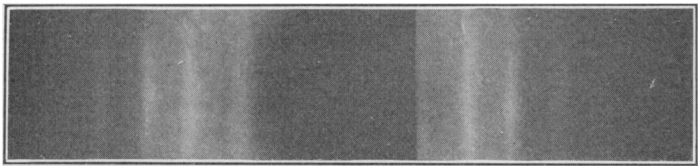


FIG. 9. Finely pulverized cane sugar.

graph. The lines are fully as strong and even clearer in this, and the exposure was fully 20 percent less than in the previous cane-sugar picture (fig. 5). The first and obvious conclusion concerning the starch is that the lines produced by the whole grains are due to the structure of the grain. The second conclusion is that the structure is neither crystalline nor amorphous.

Before speculating further on a possible structure of the whole grain, it is interesting and suggestive to compare with this work on starch some recent work done on wood and cotton cellulose fibers. In 1920, Herzog and Jancke (38) published a table of figures which they obtained by exposing wood and cotton cellulose to X-rays, in a similar manner to that which has just been described for starch. They published only the values of $(\sin \theta)/2$. These have been converted into ratios, and are given in table 8 along with those for starch.

TABLE 8. *Comparison of Ratios of Atomic Spacings for Cellulose and Starch*

Cellulose			Computed Values of Cube	Starch		
Cotton	Ramie	Wood		Potato	Cassava	Corn
1.00	1.00	1.00	1.00	1.00	1.00	1.00
.670	.677	.682	.707	.745	.758	.765
.557	.558	.554	.577	.575	.587	.592
.486	.499	.496	.500	.507	.530	.524
.443	.448	.443	.447	.452	.465	.456
.366	.367	.372	.408	.404		.416
.339	.346	.343	.354			.387

One of the very few well-established facts about starch is that it is formed only when in contact with a protoplasmic body. Likewise, cell walls are formed only when in contact with protoplasm. Cellulose and starch have long been recognized as being chemically very closely related. Here in table 8 appears again evidence of this close relationship; also there appears a very consistent difference, all the values for starch being above the corresponding ones for a cubical arrangement, while all those for cellulose are below. This would indicate a slight difference in the atomic

arrangement. The fact that both are formed at the contact surface of protoplasm favors the idea that the layers of atoms are parallel to the surface, whether curved or plane.

In conclusion, two points seem to stand out as fairly definitely proved: (1) that there is, within limits, a regular and fairly uniform arrangement of atoms in the starch grain; and (2) that this regularity is destroyed by crushing the grain, which leads to the additional statement that the regularity is not that of crystalline structure. These statements oppose the spherocrystal theory of Schimper (30) and Meyer (3), while they favor the assumption made by Kabsch (23) in 1863 that the grains have a physical uniformity which, however, is not sufficiently regular to assign crystallographic axes to them. Or it may be that, instead of planes such as are found in crystals, the regularity takes the form of curved layers.

It is a pleasure to record here an indebtedness to Dr. George J. Pierce of the Department of Botany, and to Dr. David L. Webster and Dr. Frank C. Hoyt of the Department of Physics, for helpful assistance and suggestions.

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BIBLIOGRAPHY

1. Poggendorff, J. C. *Annalen Phys. Chem.* 37: 114-160. 1836.
2. Nägeli, C. *Die Stärkekörner*. Pp. 624, 16 plates. Zürich, 1858.
3. Meyer, A. *Untersuchungen über die Stärkekörner*. Pp. 318, 9 plates. Jena, 1895.
4. Reichert, E. T. The differentiation and specificity of starches, pts. I and II. Pp. 1250, 102 plates. Carnegie Inst. Wash. Pub. 173. 1913.
5. Leeuwenhoek, A. *Opera omnia*, vol. 4. *Epistolae physiologicae* . . . Delphis, apud Adrianum Beman, p. 232. 1719. (Quoted by Payen in *Ann. Sci. Nat. Bot.* II, 10: 8, 1838.)
6. Crüger, H. *Bot. Zeit.* 12: 41-50. 1854.
7. Schimper, A. F. W. *Bot. Zeit.* 52: 884-886. 1880.
8. Strasburger, E. *Über den Bau und das Wachstum der Zellhäute*, pp. 154-159. Jena, 1882.
9. Guérin Varry. *Annales Chim. Phys.* II, 56: 225-252. 1834.
10. Raspail, F. V. *Ann. Sci. Nat.* I, 6: 384-427. 1825.
11. Whympers, R. 7th international congress of applied chemistry, sect. VIa, pp. 7-11. 1909.
12. Beijerinck, M. W. *Kon. Akad. Wetensch. Amsterdam, Proc. Sect. Sci.* 14: 1107-1110. 1912.
13. Maschke, O. *Jour. Prakt. Chem.* 56: 403. 1852.
14. Baily, W. *Philos. Mag.* V, 2: 123-126. 1876.
15. Gatin-Gruezewska, Z. *Compt. Rend. Acad. Sci. Paris* 146: 540-542. 1908.
16. Maschke, O. *Jour. Prakt. Chem.* 56: 409. 1852.
17. Nägeli, C. *Die Stärkekörner*, p. 181. Zürich, 1858.
18. Meyer, A. *Untersuchungen über die Stärkekörner*, pp. 242-246. Jena, 1895.
19. Kraemer, H. *Bot. Gaz.* 34: 352. 1902.
20. Fritzsche, J. *Ann. Phys. Chem.* 32: 131. 1834.
21. Münter, J. *Bot. Zeit.* 3: 198. 1845.
22. Kabsch, W. *Zeitschr. Anal. Chem.* 2: 216, 217. 1863.
23. Sachs, J. *Textbook of botany* (Eng. transl.), pp. 57, 58. Oxford, 1875.

25. Strasburger, E. Über den Bau und das Wachstum der Zellhäute, p. 162. Jena, 1882.
26. de Vries, H. (Abstr.) Bot. Jahresber. 13¹: 122. 1885.
27. Raspail, F. V. Ann. Sci. Nat. I, 6: 387, 388. 1825.
28. Münter, J. Bot. Zeit. 3: 194, 195. 1845.
29. Nägeli, C. Bot. Zeit. 39: 637, 638. 1881.
30. Schimper, A. F. W. Bot. Zeit. 39: 223-226. 1881.
31. Nägeli, C. Sitzungsber. Bayer. Akad. Wiss. 8: 206. 1862.
32. Rosenbusch, H. Mikroskopische Physiographie der petrographisch wichtigen Mineralen, 2d ed., pp. 33, 65. Stuttgart, 1885.
33. Fox Talbot. Trans. Roy. Soc. Edinburgh 20: 607. 1853. (Quoted by Brewster.)
34. Brewster, D. Trans. Roy. Soc. Edinburgh 20: 607. 1853.
35. Fischer, H. Beitr. Biol. Pflanz. 8: 74. 1898.
36. Strecker. Unpublished statement quoted by Czapek. Biochemie der Pflanzen, 2d ed. 1: 402. Jena, 1913.
37. Czapek, F. Biochemie der Pflanzen, 2d ed. 1: 402. 1913.
38. Herzog, R. O., and Jancke, W. Zeitschr. Physik 3³: 196, 197. 1920.
39. Bravais. Quoted by Laue. Ann. Physik 41: 971. 1913.
40. Laue, M. Ann. Physik 41: 971-978, 989-1002. 1913.
41. Friedrich, W., and Knipping, P. Ann. Physik 41: 978-988. 1913.
42. Hull, A. W. Phys. Rev. 10: 661-696. 1917.
43. Debye, P., and Scherrer, P. Phys. Zeitschr. 17: 277-282. 1916.
44. Bain, E. C. Chem. Metallurg. Engin., Oct. 5, 1921, pp. 657-664.
45. Bragg, W. H., and Bragg, W. L. X-rays and crystal structure. Pp. 230. London, 1918.
46. Hull, A. W. Phys. Rev. 17: 571-574. 1921.